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# **Accuracy of prenatal diagnosis of X-linked hypohidrotic ectodermal dysplasia by tooth germ sonography**

**Short title: XLHED diagnosis *in utero***

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## **Key words**

genetic defects, ectodermal dysplasia, tooth germ, sonography, prenatal diagnosis, facial abnormalities

## **Abstract**

**Objective:** X-linked hypohidrotic ectodermal dysplasia (XLHED), a developmental disorder characterized by malformation of hair, teeth, and sweat glands, results from defective ectodysplasin A1 (EDA1) caused by *EDA* mutations. Inability to sweat, the major problem of XLHED which can lead to life-threatening hyperthermia, has been shown to be amenable to intrauterine therapy with recombinant EDA1. The aim of this retrospective study was to evaluate the diagnostic accuracy of tooth germ sonography as a non-invasive means to identify affected fetuses in pregnant women with *EDA* mutations.

**Methods:** Tooth germ sonography was performed in 38 cases at 10 study sites between gestational weeks 18 and 28. XLHED was diagnosed if fewer than six tooth germs were detected in mandible and/or maxilla. In all subjects, diagnoses were verified postnatally by *EDA* sequencing and/or clinical findings (standardized clinical assessments of hair, sweating, and dentition; panoramic radiographs). Estimated weights of 12 affected male fetuses and postnatal weight gain of 12 boys with XLHED were assessed using appropriate growth charts.

**Results:** In 19 of 38 sonographic examinations of 23 male and 13 female fetuses, a prenatal diagnosis of XLHED was made. The diagnosis proved to be correct in 37 cases; one affected male fetus was missed. Specificity and positive predictive value were both 100%. Tooth counting by clinical assessment corresponded well with radiographic findings. We observed no weight deficits of subjects with XLHED *in utero* but occasionally during infancy.

**Conclusions:** Tooth germ sonography is highly specific and reliable in establishing a prenatal diagnosis of XLHED.

## Introduction

X-linked hypohidrotic ectodermal dysplasia is caused by mutations of the X-chromosomal gene *EDA* that lead to absence or dysfunction of the signaling protein EDA1 and thus to developmental defects of hair, teeth, and various eccrine glands including sweat glands<sup>1-3</sup>. Male subjects with XLHED suffer from oligodontia, hypo- or anhidrosis with very dry, often eczematous skin, dry eyes, and recurrent respiratory infections<sup>4-8</sup>. Inability to sweat poses affected individuals at risk of life-threatening hyperthermia, especially in early infancy<sup>4,8-10</sup>. Missing or peg-shaped teeth are also of clinical relevance<sup>11,12</sup>, as they may cause mastication problems and reduced nutritional intake. Failure to thrive has been observed in many affected boys<sup>13</sup>. Mortality is high (2 to 30%) and depends on the time point of diagnosis<sup>4,10</sup>.

So far, treatment of XLHED has been symptomatic. New therapeutic options may arise from a recombinant EDA1 molecule which rescued normal development in murine and canine models of XLHED<sup>14-16</sup>. In order to achieve the same therapeutic effects in humans, prenatal administration of the EDA1 replacement protein appears to be required<sup>17,18</sup>. Three recent case studies have shown that intra-amniotic injection of recombinant EDA1 at the beginning of the third trimester of pregnancy may prevent the development of XLHED-related morbidity<sup>18</sup>. A clinical trial to evaluate this therapeutic approach is currently being prepared. For such treatment *in utero*, however, a reliable low-risk method to diagnose XLHED prenatally will be indispensable.

Ultrasound is currently the standard non-invasive prenatal diagnostic tool; tooth buds and thus oligodontia can be visualized already in the second trimester of pregnancy<sup>19,20</sup>. Tooth germ sonography or sonographic screening for facial characteristics has been used in single centers to identify fetuses with XLHED<sup>21,22</sup>. The aim of this retrospective multi-center study was to evaluate the accuracy of tooth germ sonography as a non-invasive method to establish a prenatal diagnosis of XLHED in pregnant carriers of *EDA* mutations.

## Subjects and methods

### Prenatal sonography

Ten prenatal medicine specialist sites in Germany and Great Britain participated in the study. Physicians with profound experience in fetal sonography carried out the ultrasound examinations using standard high-end devices. A total of 33 pregnant women with known *EDA* mutations, one with a polyzygotic triplet pregnancy, one with a monozygotic twin pregnancy, and 31 with singleton pregnancies, were referred to one of the participating sites in the years 2010–2017. Detailed sonography was carried out at a gestational age of 18–28 weeks. All women gave informed consent prior to the ultrasound scan. Two pregnant women underwent independent ultrasound examinations at two different sites; observers were blinded to the previous findings so that a prenatal diagnosis was established in 38 cases.

In two-dimensional axial sections of mandible and maxilla, round hypoechogenic structures with a hyperechogenic margin were identified as dental alveoli and counted; their number was considered to be reduced if it was below six in one of the alveolar bones<sup>20,22</sup>. Additional screening for fetal malformations was performed. Based on the tooth germ counts we diagnosed or excluded fetal XLHED. In a few cases, the number of tooth germs was not determined exactly, but classified as “reduced” or “normal”. All prenatal diagnoses were verified by *EDA* gene sequencing (n=26) and/or clinical findings including standardized assessments of hair, sweating, and dentition after birth (n=31).

### Postnatal tooth quantification

This investigation was part of the clinical study “Natural history and outcomes in X-linked hypohidrotic ectodermal dysplasia” (NCT02099552, [www.clinicaltrials.gov](http://www.clinicaltrials.gov)). Parents or legal guardians provided informed consent to the study procedures which had been approved by an independent institutional ethics committee and were conducted according to national regulations and GCP/ICH guidelines. Subjects were included only if pathogenic *EDA* mutations had been detected. Standardized clinical assessments of dentition in children were compared with panoramic radiographs () where the number of teeth and tooth buds was determined by experienced dentists. Panoramic radiographs (or “x-rays”) from the XLHED patients reported here were obtained at the age of 4 7/12 to 5 1/12 years.

#### Assessment of pre- and postnatal weight gain

Based on measurements of abdominal circumference, femur length, and head circumference, the body weight of 12 male fetuses with XLHED was estimated according to the formula used by default at each site (in most cases the formula of Hadlock et al<sup>23</sup>). Percentiles for the control population were taken from published WHO fetal growth charts<sup>24</sup>.

Biometrical data obtained during routine pediatric examinations were included in the retrospective analysis of weight-for-age charts with published percentiles for the normal population<sup>25</sup>. For preterm-infants, only age-corrected data were used.

## Results

Prenatal tooth germ sonography at 10 study sites in Germany and Great Britain led to the prenatal diagnosis of XLHED in 19 of 38 cases (Table 1). Most of these investigations were carried out at one site, a level III university hospital; 13 examinations were performed at the nine other sites.

A partial set of data on fetuses M1–M7 and F1–F4 was published previously<sup>22</sup>. In all sonographic examinations, tooth-bearing bones were depictable in axial two-dimensional ultrasound sections and dental alveoli were evaluated in both jaws (Fig. 1). Lack of tooth germs was observed more often in the mandible than in the maxilla. In eight male fetuses, tooth buds were completely absent in the lower jaw (Table 2). A reduced number of tooth germs was typically accompanied by a thin, hypoplastic alveolar bone (Fig. 1). Ten male and nine female fetuses, each with six or more tooth germs both in maxilla and mandible, were considered as not affected (Table 2). Molecular genetic investigations and/or postnatal clinical assessments of dentition (Fig. 2) confirmed the prenatal diagnosis in 37 of 38 cases. In one subject, the prenatal diagnosis proved to be wrong: a male fetus with sonographically normal appearance of tooth germs was considered as not affected, but XLHED was diagnosed postnatally (Table 2).

In total, 19 of 20 XLHED subjects were identified prenatally by tooth germ sonography (sensitivity of 95.0%). There was no false positive result, so that specificity and positive predictive value of prenatal XLHED diagnosis by tooth germ sonography were both 100%. The prenatal diagnosis “not affected subject” proved to be right in 18 of 19 cases; the negative predictive value was 94.7%. Overall, prenatal diagnosis was correct in 97.4% of cases (Tables 1 and 2).

Pre- and postnatal assessments of tooth endowment usually corresponded well. In patient M6, for example, five tooth germs had been identified in the maxilla prenatally, six were detected radiographically at an age of 4 years (Fig. 3); the three tooth germs counted in the mandible of that patient *in utero* were confirmed to be three tooth germs in the panoramic radiographs (Fig. 3). Prenatal sonography of patient M7 had revealed two tooth germs in the maxilla and none in the mandible, while later three teeth were found in the maxilla and none in the mandible (Table 3). In patient F2, however, the number of tooth germs recognized prenatally was clearly

reduced (four tooth germs in the maxilla, five in the mandible). Postnatally, eight deciduous and nine permanent teeth were seen in the maxilla as well as 10 deciduous and 12 permanent teeth in the mandible. Thus, not all tooth germs were detectable prenatally, but all tooth germs identified by ultrasonography *in utero* corresponded to teeth/tooth germs visible later in a dental radiograph.

Comparison of the results of tooth counting by clinical assessment in pre-school children with later radiographic findings showed good agreement (Table 3). In 14 male and five female XLHED subjects aged 4.5 to 5 years, the number of teeth was clearly more diminished in male patients, especially in the mandible. It ranged from two to six deciduous and nil to four permanent teeth in the maxilla and from nil to six deciduous and nil to two permanent teeth in the mandible. The mean number of teeth also differed between mandible and maxilla (4.1 deciduous and 2.1 permanent teeth in the maxilla, 1.5 deciduous and 0.6 permanent teeth in the mandible). Dentition was usually delayed. Many of the teeth had dysmorphic crowns: conical teeth were observed very often. Some teeth were horizontally inclined, especially deciduous teeth without permanent successor (Figs. 2 and 3). Taurodontism and taproots (Fig. 3) occurred frequently. As observed in the prenatal scans, a reduced number of teeth/tooth buds was accompanied by obvious alveolar hypoplasia (Fig. 2).

Most female subjects with XLHED also had a considerably reduced tooth endowment (mean numbers of 7.8 deciduous and 10.0 permanent teeth in the maxilla and 8.4 deciduous and 11.0 permanent teeth in the mandible). The number of teeth in female subjects, however, showed great inter-individual variability (Table 3). Even if it was normal, some of the teeth had morphological abnormalities as observed in male XLHED subjects.

To find out whether the reported weight deficits of boys with XLHED are acquired pre- or postnatally, estimated fetal weights and postnatal weight gain were assessed using weight-for-age charts. The estimated weight of 12 male fetuses with a prenatal diagnosis of XLHED was mostly between the 25<sup>th</sup> and the 75<sup>th</sup> percentile of WHO growth charts for the respective gestational age<sup>24</sup>; none of the estimated fetal weights fell below the 10<sup>th</sup> percentile. Thus, the weight of male fetuses with XLHED was not diminished (Fig. 4). In the group of 12 male subjects who had already completed the XLHED natural history study, delineation of biometric data in growth charts<sup>25</sup> showed



that their weight for age was mostly below average. At the age of five years, the weight of only two subjects was above the 50<sup>th</sup> percentile, whereas four of 12 subjects (three of them with very few teeth) had a weight below the 10<sup>th</sup> percentile (Fig. 4). We conclude from these data that weight gain of XLHED subjects is impaired only postnatally.

## Discussion

This analysis of 38 cases shows that prenatal tooth germ sonography is a highly reliable method to establish a prenatal diagnosis of XLHED. It was applied to fetuses of pregnant women with known *EDA* mutations, thus in a cohort with a 50% risk of the fetus being affected, however without any false positive result. High specificity and a high positive predictive value are important prerequisites for employing a screening method in a clinical setting where affected subjects may undergo treatment. Considering the specificity of 100%, tooth germ sonography would be well suitable for identifying patients to be treated *in utero* by intra-amniotic administration of an EDA1 replacement molecule. This non-invasive method could be used for screening purposes, as it does not expose the fetus or the mother to any procedure-related risk. The approach was applicable at various sites in different countries, representing different levels of care, in university hospitals as well as in private medical centers. Prenatal screening for oligodontia can easily be integrated into ultrasound examinations that are routinely performed during pregnancy. Additional equipment is not necessary. Thus, tooth germ sonography could be made easily available and may be applied broadly to diagnose XLHED early enough for potential treatment.

Apart from this, tooth germ sonography may be offered to all pregnant women with known *EDA* mutation who desire a prenatal diagnosis. Early recognition of XLHED may be crucial for reducing the risk of overheating. Dangerous and sometimes life-threatening episodes of hyperpyrexia occur most often in young infants, because they frequently suffer febrile illnesses and cannot evade overheating by themselves<sup>4,10</sup>. Beyond infancy, males with XLHED can prevent hyperthermia by avoiding hot and sunny places, by wetting their T-shirt or by wearing cooling devices during physical activity<sup>4,26</sup>. Besides genetic testing after diagnostic amniocentesis,

measurement of the ratio of variant to normal *EDA* cell-free DNA in the maternal plasma, or genotyping of the infant using cord blood, would represent alternative methods to diagnose XLHED either pre- or peri-natally. Measurement of the EDA protein in cord blood may be another approach applicable to at least some classes of mutation<sup>27</sup>.

Tooth germ sonography may, however, be applicable more broadly. Prenatal assessment of facial features via two-dimensional ultrasound has become an important part of screening for fetal abnormalities; because oligodontia is associated with fetal pathology in a substantial number of cases, visualization of alveolar structures has been assigned a larger role in prenatal diagnostics<sup>28</sup>. Of course we recognise that in these circumstances - without a prior indication of *EDA* gene involvement - the measures of test performance reported here will not apply, because the prior probability that the cause is a pathogenic variant in *EDA* will be so much smaller.

Numerical or morphological tooth abnormalities were found in all XLHED subjects investigated postnatally. Oligodontia was more pronounced in the mandible and, as expected, in male subjects. Even if the number of teeth was not considerably reduced in some affected females, their teeth were not all normal. Taurodontism as well as teeth with tapered and conical forms are typical of XLHED<sup>11,12</sup>. Consistent with previous reports<sup>12</sup>, postnatal tooth quantification showed a wide variability of the number of teeth in XLHED females. This phenotypic variability in heterozygous carriers is characteristic of X-linked diseases and well known in XLHED<sup>4</sup>. Although in our cohort all four females with XLHED were recognized prenatally, the sensitivity of tooth germ sonography is likely to be lower in affected female subjects who do not lack many teeth, and this may represent a limitation of the method. However, the specificity is expected to be high as hypodontia in the female deciduous dentition is rare (Brook 1974) so that, when it is recognised in a situation of high prior probability of a female carrying XLHED, this makes it very likely that she will indeed be a carrier. Furthermore, the lower sensitivity of the test in the female fetus may be not as invidious as a false positive result in a fetus of either sex.

Mandibular hypoplasia is a consequence of oligodontia and was consistently observed prenatally as well as postnatally. Due to missing teeth, reduced alveolar

processes, and mandibular hypoplasia individuals with XLHED often have a typical facial appearance with a negative overjet<sup>29,30</sup>. Absent or mis-shaped teeth are frequently the reason to seek medical help for individuals with previously undiagnosed XLHED<sup>29</sup> and pose a significant psychosocial burden on the patients<sup>31</sup>. Although oligodontia is not life-threatening, it affects the quality of life and may be one of probably multiple causes of impaired postnatal weight gain as observed in our cohort of children with XLHED. Growth abnormalities with a reduced mean weight-for-age have also been observed by others<sup>13</sup>. There is no doubt that oligodontia has a negative impact on orofacial functioning and mastication. In addition to salivary gland dysfunction in XLHED<sup>32</sup>, this may prevent adequate caloric intake. Therefore, special attention should be paid to timely provision of prostheses.

In summary, oligodontia and mandibular hypoplasia are consistent and characteristic features of XLHED at least in affected male subjects that can already be detected prenatally. Tooth germ sonography is a highly reliable method to establish a prenatal diagnosis of XLHED. In the high-risk collective of pregnant women with known *EDA* mutation, specificity and positive predictive value were both 100%. This non-invasive method could thus be used regularly to recognize XLHED in time for possible prenatal therapy or prevention of dangerous hyperthermic episodes in early infancy.

## Figure legends

### **Figure 1. Prenatal diagnosis of XLHED by tooth germ sonography.**

In healthy controls (upper and lower right panel), axial sections of alveolar bones show a normal number of dental alveoli, represented by round hypoechogenic structures with hyperechogenic margins and highlighted by white arrows. In fetuses with a reduced number of tooth germs in the maxilla (upper left panel: fetus M11 at 21 weeks of gestation) or in the mandible (lower left panel: fetus M13 at 23 weeks of gestation), a prenatal diagnosis of XLHED was made. Lack of tooth germs was accompanied by a thin, hypoplastic alveolar bone.

### **Figure 2. Clinical assessment of oligodontia and alveolar hypoplasia.**

Intra-oral dental photographs depicting the frontal view **(A)** and the mandible **(B)** of a boy with XLHED. The pictures display the typical oligodontia, tooth malformation, and alveolar hypoplasia of the edentulous bone regions.. **(C)** Panoramic radiograph of the same patient confirming the presence of four teeth as well as two additional upper primary canines as well as permanent tooth germs for the present central incisors.

### **Figure 3. Comparison of prenatal tooth germ sonography and corresponding panoramic radiograph.**

**A)** A horizontal section of the maxilla of a fetus at 20 weeks of gestation (patient M6) shows only five dental alveoli, highlighted by white arrows. A prenatal diagnosis of XLHED was made. **B)** Panoramic x-ray of the same patient at the age of four years.

### **Figure 4. Body weight of male subjects with XLHED.**

**A)** The estimated weight of 12 male XLHED fetuses was set in relation to WHO fetal growth charts<sup>24</sup>. The weight of twins or triplets is indicated as X, the weight of single fetuses as triangle. For one fetus, the weight was estimated at two time points, depicted as triangles connected by a dashed line. None of the fetuses investigated had an estimated weight below the 10<sup>th</sup> percentile. **B)** For 12 boys with XLHED, the weight obtained during routine pediatric examinations was delineated in weight-for-age charts<sup>25</sup>. The weight of twins or triplets is indicated as X, the weight of children from a singleton pregnancy is indicated as triangle. In most cases, weight for age

was below the 50<sup>th</sup> percentile. Four of 12 subjects had a weight below the 10<sup>th</sup> percentile after their 4<sup>th</sup> birthday.

## References

1. Kere J, Srivastava AK, Montonen O, Zonana J, Thomas N, Ferguson B, Munoz F, Morgan D, Clarke A, Baybayan P, Chen EY, Ezer S, Saarialho-Kere U, de la Chapelle A, Schlessinger D. X-linked anhidrotic (hypohidrotic) ectodermal dysplasia is caused by mutation in a novel transmembrane protein. *Nat Genet* 1996; **13**: 409–416.
2. Mikkola ML. Molecular aspects of hypohidrotic ectodermal dysplasia. *Am J Med Genet A* 2009; **149A**: 2031–2036.
3. Visinoni AF, Lisboa-Costa T, Pagnan NA, Chautard-Freire-Maia EA. Ectodermal dysplasias: clinical and molecular review. *Am J Med Genet A* 2009; **149A**: 1980–2002.
4. Clarke A, Phillips DI, Brown R, Harper PS. Clinical aspects of X-linked hypohidrotic ectodermal dysplasia. *Arch Dis Child* 1987; **62**: 989–996.
5. Itin PH, Fistarol SK. Ectodermal dysplasias. *Am J Med Genet C Semin Med Genet* 2004; **131C**: 45–51.
6. Schneider H, Hammersen J, Preisler-Adams S, Huttner K, Rascher W, Bohring A. Sweating ability and genotype in individuals with X-linked hypohidrotic ectodermal dysplasia. *J Med Genet* 2011; **48**: 426–432.
7. Dietz J, Kaercher T, Schneider AT, Zimmermann T, Huttner K, Johnson R, Schneider H. Early respiratory and ocular involvement in X-linked hypohidrotic ectodermal dysplasia. *Eur J Pediatr* 2013; **172**: 1023–1031.
8. Fete M, Hermann J, Behrens J, Huttner KM. X-linked hypohidrotic ectodermal dysplasia (XLHED): clinical and diagnostic insights from an international patient registry. *Am J Med Genet A* 2014; **164A**: 2437–2442.
9. Salisbury DM, Stothers JK. Hypohidrotic ectodermal dysplasia and sudden infant death. *Lancet* 1981; **1(8212)**: 153–154.
10. Blüschke G, Nüsken KD, Schneider H. Prevalence and prevention of severe complications of hypohidrotic ectodermal dysplasia in infancy. *Early Hum Dev* 2010; **86**: 397–399.
11. Präger TM, Finke C, Miethke RR. Dental findings in patients with ectodermal dysplasia. *J Orofac Orthop* 2006; **67**: 347–355.
12. Lexner MO, Bardow A, Hertz JM, Nielsen LA, Kreiborg S. Anomalies of tooth formation in hypohidrotic ectodermal dysplasia. *Int J Paediatr Dent* 2007; **17**: 10–18.
13. Motil KJ, Fete TJ, Fraley JK, Schultz RJ, Foy TM, Ochs U, Sybert VP. Growth characteristics of children with ectodermal dysplasia syndromes. *Pediatrics* 2005; **116**: e229–234.

14. Gaide O, Schneider P. Permanent correction of an inherited ectodermal dysplasia with recombinant EDA. *Nat Med* 2003; **9**: 614–618.
15. Casal ML, Lewis JR, Mauldin EA, Tardivel A, Ingold K, Favre M, Paradies F, Demotz S, Gaide O, Schneider P. Significant correction of disease after postnatal administration of recombinant ectodysplasin A in canine X-linked ectodermal dysplasia. *Am J Hum Genet* 2007; **81**: 1050–1056.
16. Hermes K, Schneider P, Krieg P, Dang A, Huttner K, Schneider H. Prenatal therapy in developmental disorders: drug targeting via intra-amniotic injection to treat X-linked hypohidrotic ectodermal dysplasia. *J Invest Dermatol* 2014; **134**: 2985–2987.
17. Huttner K. Future developments in XLHED treatment approaches. *Am J Med Genet A* 2014; **164A**: 2433–2436.
18. Schneider H, Faschingbauer F, Schuepbach-Mallepell S, Körber I, Wohlfart S, Dick A, Wahlbuhl M, Kowalczyk-Quintas C, Vigolo M, Kirby N, Tannert C, Rompel O, Rascher W, Beckmann MW, Schneider P. Prenatally administered drug targeting genetic developmental disorder. *New Engl J Med* 2018, in press.
19. Ulm MR, Chalubinski K, Ulm C, Plöckinger B, Deutinger J, Bernaschek G. Sonographic depiction of fetal tooth germs. *Prenat Diagn* 1995; **15**: 368–372.
20. Ulm MR, Kratochwil A, Ulm B, Solar P, Aro G, Bernaschek G. Three-dimensional ultrasound evaluation of fetal tooth germs. *Ultrasound Obstet Gynecol* 1998; **12**: 240–243.
21. Sepulveda W, Sandoval R, Carstens E, Gutierrez J, Vasquez P. Hypohidrotic ectodermal dysplasia: prenatal diagnosis by three-dimensional ultrasonography. *J Ultrasound Med* 2003; **22**: 731–735.
22. Wünsche S, Jüngert J, Faschingbauer F, Mommsen H, Goecke T, Schwanitz K, Stepan H, Schneider H. Noninvasive Prenatal Diagnosis of Hypohidrotic Ectodermal Dysplasia by Tooth Germ Sonography. *Ultraschall Med* 2015; **36**: 381–385.
23. Hadlock FP, Harrist RB, Carpenter RJ, Deter RL, Park SK. Sonographic estimation of fetal weight. The value of femur length in addition to head and abdomen measurements. *Radiology* 1984; **150**: 535–540.
24. Kiserud T, Piaggio G, Carroli G, Widmer M, Carvalho J, Neerup Jensen L, Giordano D, Cecatti JG, Abdel Aleem H, Talegawkar SA, Benachi A, Diemert A, Tshefu Kitoto A, Thinkhamrop J, Lumbiganon P, Tabor A, Kriplani A, Gonzalez Perez R, Hecher K, Hanson MA, Gülmezoglu AM, Platt LD. The World Health Organization Fetal Growth Charts: A Multinational Longitudinal Study of Ultrasound Biometric Measurements and Estimated Fetal Weight. *PLoS Med* 2017; **14**: e1002220.
25. Neuhauser H, Schienkiewitz A, Schaffrath Rosario A, Dortschy R, Kurth BM. *Referenzperzentile für anthropometrische Maßzahlen und Blutdruck aus der*

*Studie zur Gesundheit von Kindern und Jugendlichen in Deutschland (KiGGS). Beiträge zur Gesundheitsberichterstattung des Bundes. 2nd edition. Robert Koch-Institut, Berlin, 2013; 22–31.*

26. Hammersen JE, Neukam V, Nüsken KD, Schneider H. Systematic evaluation of exertional hyperthermia in children and adolescents with hypohidrotic ectodermal dysplasia: an observational study. *Pediatr Res* 2011; **70**: 297–301.
27. Podzus J, Kowalczyk-Quintas C, Schuepbach-Mallepell S, Willen L, Staehlin G, Vigolo M, Tardivel A, Headon D, Kirby N, Mikkola ML, Schneider H, Schneider P. Ectodysplasin A in Biological Fluids and Diagnosis of Ectodermal Dysplasia. *J Dent Res* 2017; **96**: 217–224.
28. Seabra M, Vaz P, Valente F, Braga A, Felino A. Two-Dimensional Identification of Fetal Tooth Germs. *Cleft Palate Craniofac J* 2017; **54**: 166–169.
29. Bergendal B. Oro dental manifestations in ectodermal dysplasia-a review. *Am J Med Genet A* 2014; **164A**: 2465–2471.
30. Hadj-Rabia S, Schneider H, Navarro E, Klein O, Kirby N, Huttner K, Wolf L, Orin M, Wohlfart S, Bodemer C, Grange DK. Automatic recognition of the XLHED phenotype from facial images. *Am J Med Genet A* 2017; **173**: 2408–2414.
31. Saltnes SS, Jensen JL, Sæves R, Nordgarden H, Geirdal AØ. Associations between ectodermal dysplasia, psychological distress and quality of life in a group of adults with oligodontia. *Acta Odontol Scand* 2017; **75**: 564–572.
32. Lexner MO, Bardow A, Hertz JM, Almer L, Nauntofte B, Kreiborg S. Whole saliva in X-linked hypohidrotic ectodermal dysplasia. *Int J Paediatr Dent* 2007; **17**: 155–162.

#### POSSIBLE ADDITIONAL REFERENCE

Brook AH. Dental anomalies of number, form and size: their prevalence in British school children. *7 Int Assoc Dent Child* 1974; 5:37-53.